Supporting Information

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SI Methods

Baseline Homecage Behavior. Mice were placed individually into behavioral testing chambers. Activity was assessed using behavior monitoring equipment which collects movements as number of beam breaks. The infra-red photobeams cover the cage area in a 1 cm, 3D grid (AccuScan Instruments). Disruption of a beam results in an activity count, which is recorded in a PC using Versamax software (AccuScan Instruments). Movement measurements included vertical activity (VACT), horizontal activity (HACT), and total distance (TD).

Sensory Alertness. Sensory alertness was measured via amount of vertical activity collected in the behavioral testing chambers described above (for home cage activity measures). Hardware (LabLinc V; Coulbourn Instruments) controlled stimulus delivery directed by Graphic State software (Coulbourn Instruments). This allowed for stimulus delivery and data collection without any experimenter interruption.

Sensory testing commenced 1 h after lights on. The automated stimulus delivery program required 5 min of resting state, defined as no horizontal movement before delivering any stimulus. The tactile stimulus was delivered first. A 10 s puff of air at 16 psi was delivered through nozzles positioned at the top corners of the cage. An obligatory waiting period of at least 10 min was required of each animal before the vestibular stimulus was delivered. The vestibular stimulus was delivered by activation of the orbital shakers placed underneath each behavioral testing chamber for 15 s at 90 rpm. After the animal returned to a resting state and at least 10 min passed, the olfactory stimulus was delivered last. This consisted of a 10 s stream of air passing through a jar filled with benzaldehyde (an almond smell, Sigma) and into the cage through two jets placed near the floor.

Startle. Testing was conducted in an SR-Lab System enclosed in an isolation chamber ($15 \times 16 \times 23$ inches). Animals were placed in a clear Plexiglas cylinder (5.1 cm OD, 12 cm long) on a piezoelectric vibration sensor. The sensor was connected to a PC for stimulus delivery and data acquisition. A speaker placed 28 cm above the cylinder emitted sound levels calibrated by a sound level meter. Animals were run in the startle test between 2 and 4 hours after lights off. Before each test commenced, a 5 min acclimation period allowed the animals to adjust to the environment and the 86 dB background white noise.

In the startle test, each subject was presented a total of 90 stimuli, each lasting 40 ms over a 25 min period. Nine different sound levels (82, 88, 92, 96, 100, 105, 110, 115, 120 dB) were presented in a pseudorandom order in 10 blocks. The intertrial interval was randomized between 9 and 23 s to prevent habituation. Movement was recorded by the sensor every 1 ms for 100 ms following the onset of the each sound stimulus. The maximum startle amplitude, latency to the startle amplitude and average amplitude were all recorded.

Tail Flick. Pain threshold was measured using a Tail Flick Monitor (Model TF, Omnitech). Briefly, the mice were held stationary on the platform and the tail inserted into the groove before the heating device was turned on. The latency to the tail flick off of the heating apparatus was determined by laser beams.

Assays. Animals were killed at lights off by cervical dislocation and then decapitation. Brains were removed from crania and snap frozen in 1.5 ml centrifuge tubes and then stored at −80°C until processing. Brain tissue was allowed to thaw and then homogenized in a 1% SDS solution in d_2H_2O with protease inhibitor (Roche Diagnostics). The homogenate was spun down for 30 min at 4°C. The supernatant was removed and stored at −80 °C until processing. Homogenate supernatant was assayed according to manufacturer direction using commercially available Histamine EIA kit (Cayman Chemicals).

Toluidine Blue Stain for Mast Cells. Following perfusion and postfixation with 4% paraformaldehyde, brains were placed in 20% sucrose for 48 h. Brains were cut on a cryostat at 40 μm and mounted onto gel-subbed slides. Sections were then subject to staining with toluidine blue (Sigma-Aldrich), dehydrated, and coverslipped for microscopy. Images were taken using an Olympus BH2 microscope. Auto color was applied in Photoshop for enhancement of image.

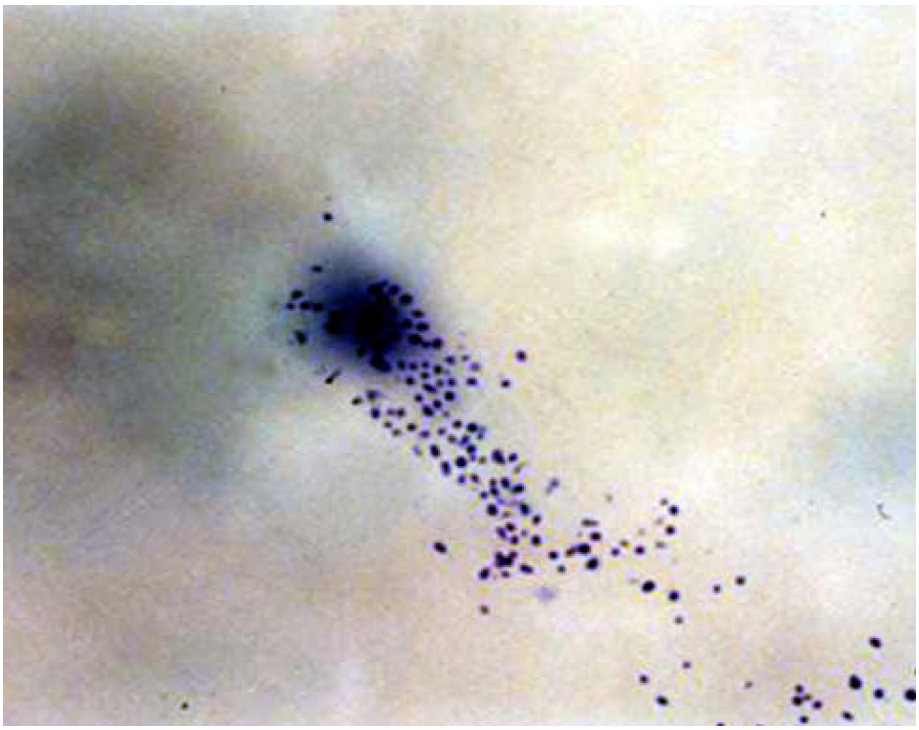

Fig. S1. The photomicrograph shows a single degranulating mast cell in the mouse brain stained with toluidine blue. Granule remnants are seen at a distance of over 50 microns from the cell body.

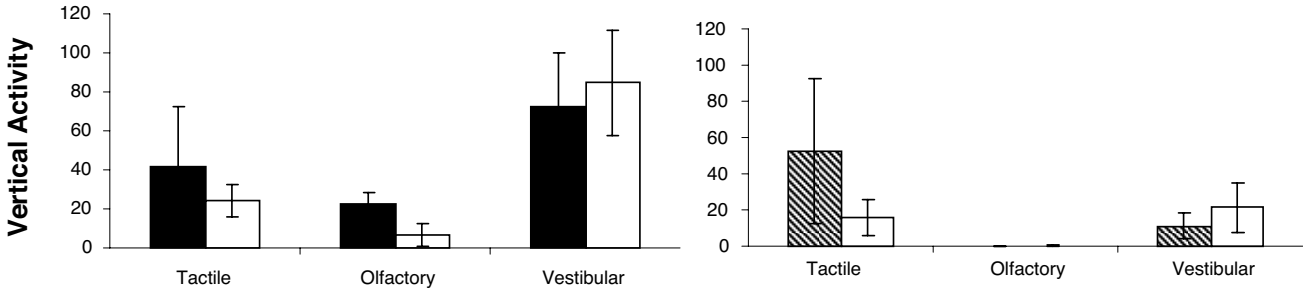

Fig. S2. Sensory alertness. There were no significant differences in the arousal responses of sash$^{-/-}$ mice (white bars) compared to WT (black bars; *Left*) or sash$^{+/-}$ littermate (hatched bars; *Right*) controls. Vertical movement measures of activity are shown for each experimental run in response to tactile, olfactory, and vestibular stimuli. Data are shown as mean ± SEM.

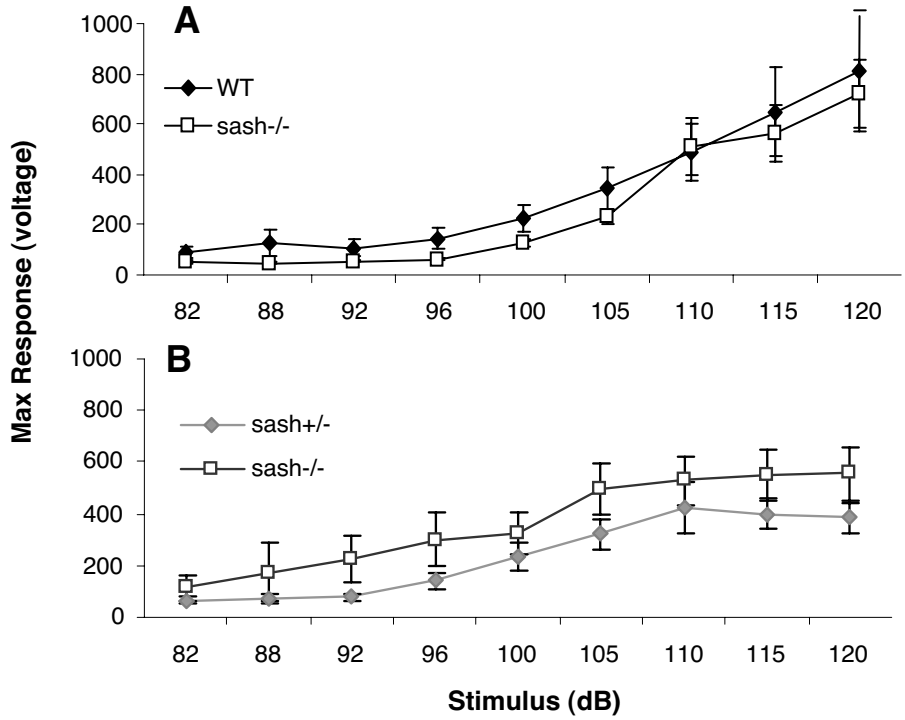

Fig. S3. Startle reflex. There were no significant differences in the startle reflex to auditory tones between sash$^{-/-}$ mice (white rectangles) and WT (black diamonds; *A*) or sash$^{+/-}$ littermate (gray diamonds; *B*) controls. The maximum response (volts) is shown as mean ± SEM to various startle tones.

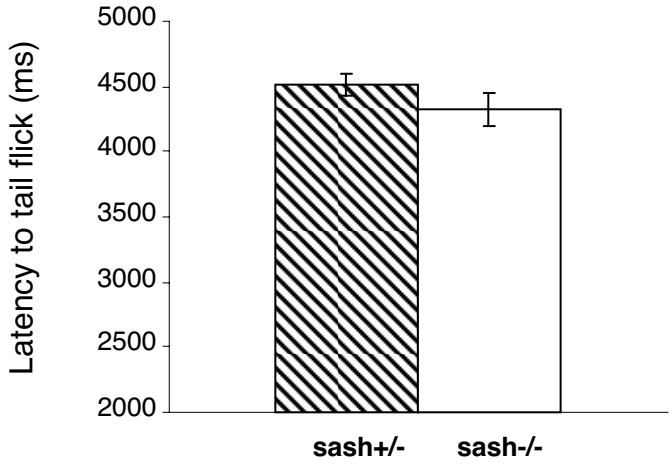

Fig. S4. Pain threshold. There were no significant differences between sash$^{-/-}$ (white bar) and sash$^{+/-}$ littermate controls (hatched bar) in the latency to flick their tail away from a heated coil. Tail flick latency (ms) is represented as mean ± SEM.

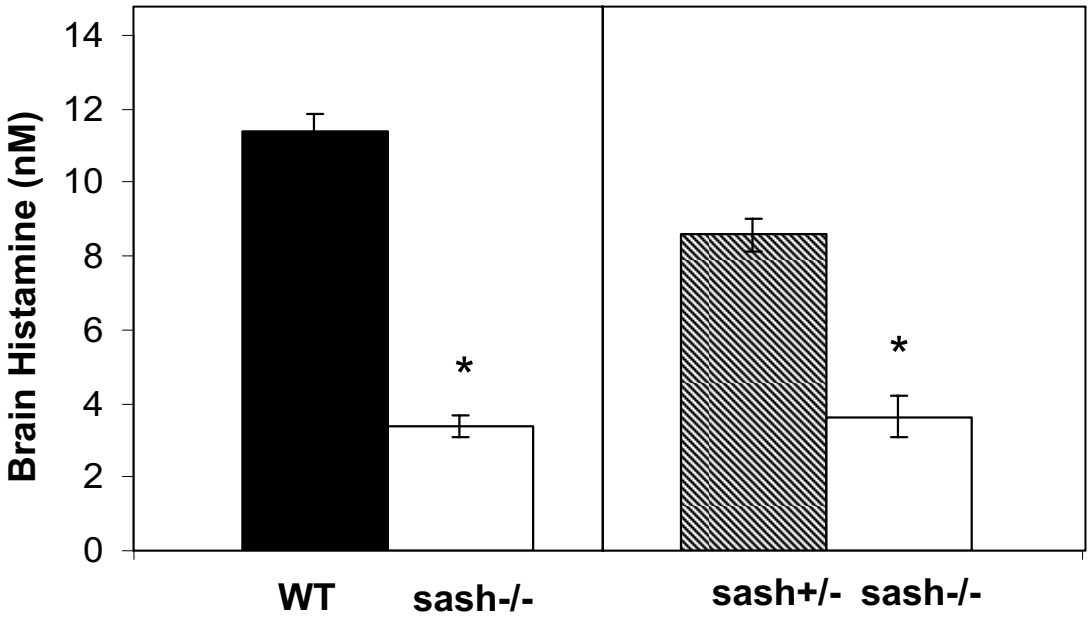

Fig. S5. Histamine measures of whole brain homogenates. (*A*) Sash$^{-/-}$ mice (white bar) have 68.4% less brain histamine than WT controls (black bar). (*B*) Sash$^{-/-}$ mice (white bar) have 58.1% less brain histamine compared to their sash$^{+/-}$ littermate controls (hatched bar). Histamine levels are represented as mean ± SEM.